

WHAT IS CLAIMED:

1. A method for the differentiation of genetic sub-types within a population of genetically related organisms comprising the steps of:
 - (i) isolating genomic DNA from an organism to be analyzed;
 - 5 (ii) restricting genomic DNA from said organism with at least one restriction endonuclease to yield restricted DNA wherein all fragments are less than 18kb;
 - (iii) resolving said restricted DNA by chromatographic means capable of separating fragments in the 0.1 to 18 kb range to generate a distinct genetic
10 fingerprint;
 - (iv) comparing said distinct genetic fingerprint of step (iii) with the genetic fingerprint of a specific genetic sub-type wherein differences in said genetic fingerprints afford differentiation of genetic sub-types within a population of genetically related organisms.
- 15 2. A method according to Claim 1 wherein said organism is selected from the group consisting of prokaryotes and microbial eukaryotes.
3. A method according to Claim 2 wherein said organism is selected from the group consisting of gram positive bacteria and gram negative bacteria.
4. A method according to Claim 3 wherein said organism is selected from
20 the group consisting of *Escherichia*, *Salmonella*, *Shigella*, *Vibrio*, *Listeria*, *Staphylococcus*, *Streptococcus*, *Enterococci*, *Klebsiella*, *Nisseria*, *Pseudomonas* and *Campylobacter*.
5. A method according to Claim 4 wherein said organism is selected from the group consisting of *E. coli* 0157 and *Salmonella*.
- 25 6. A method according to Claim 1 wherein said chromatographic means of step (iii) is selected from the group consisting of gel electrophoresis, HPLC, flow cytometry, and membrane chromatography.
7. A method according to Claim 6 wherein said method of gel electrophoresis is selected from the group consisting of capillary gel electrophoresis,
30 pulsed field gel electrophoresis, 2 dimensional gel electrophoresis, and field inversion electrophoresis.
8. A method according to Claim 7 wherein said gel is an agarose gel having a concentration of from about 0.6% to about 2% agarose.
9. A method according to Claim 7 wherein said gel is polyacrylamide.
- 35 10. A method according to Claim 7 wherein said restricted DNA is stained with a DNA binding dye selected from the group consisting of ethidium bromide, propidium iodide, and fluorescent cyanine dyes.

REPLACEMENT SHEET

11. A method according to Claim 1 wherein said at least one restriction enzyme is selected from the group consisting of *AccI*, *AluI*, *AvaI*, *AvaII*, *BanII*, *BfaI*, *CfoI*, *DdeI*, *DpnI*, *HaeII*, *HaeIII*, *HhaI*, *HincII*, *HinfI*, *HpaII*, *MboI*, *MnII*, *MseI*, *NciI*, *NlaIV*, *RsaI*, *Sau3AI*, *ScrFI*, *TaqI*, and *ThaI*.
- 5 12. A method according to Claim 1 wherein said at least one restriction enzyme is a 4 base cutter.
13. A method according to Claim 1 wherein said at least one restriction enzyme is a combination of at least two 6 base cutters.
14. A method according to Claim 1 wherein said genomic DNA is from
10 about 5 ug to about 30 ug.
15. A method according to Claim 1 wherein said resolving of restricted DNA at step (iii) results in the elimination of all DNA fragments less than 2 kb.
16. A method for the interstrain differentiation of bacterial isolates comprising the steps of:
- 15 (i) isolating genomic DNA from a) a panel of epidemiologically linked bacterial isolates and b) a panel of epidemiologically un-linked bacterial isolates wherein said linked and un-linked isolates are members of the same genus;
- (ii) restricting genomic DNA from the linked and un-linked panel of isolates with a panel of restriction enzymes selected from the group consisting of
20 *AccI*, *AluI*, *AvaI*, *AvaII*, *BanII*, *BfaI*, *CfoI*, *DdeI*, *DpnI*, *HaeII*, *HaeIII*, *HhaI*, *HincII*, *HinfI*, *HpaII*, *MboI*, *MnII*, *MseI*, *NciI*, *NlaIV*, *RsaI*, *Sau3AI*, *ScrFI*, *TaqI*, and *ThaI*;
- (iii) resolving the restricted DNA of step (ii) by gel electrophoresis to generate a banding pattern for each enzyme;
- (iv) selecting at least one enzyme from the panel of enzymes of step (ii)
25 that a) restricts all of the genomic DNA to fragments of less than 18 kb, b) generates the lowest index of diversity from the linked panel and c) generates the highest index of diversity from the unlinked panel;
- (v) restricting the isolated DNA from the linked and un-linked panels of bacterial isolates of step (i) with the at least one enzyme of step (iv);
- 30 (vi) resolving said restricted DNA of step (v) by gel electrophoretic means capable of separating fragments in the 0.1 to 18 kb range to generate a distinct genetic fingerprint for said linked and unlinked isolates; and
- (vii) comparing said distinct genetic fingerprints of step (vi) wherein differences in said genetic fingerprints affords interstrain differentiation of said
35 bacterial isolates.
17. A method according to Claim 16 wherein said method of gel electrophoresis is selected from the group consisting of, capillary gel electrophoresis,

pulsed field gel electrophoresis, 2 dimensional gel electrophoresis, inversional electrophoresis and vertical electrophoresis.

18. A method according to Claim 16 wherein said gel is an agarose having a concentration of from about 0.6% to about 2% agarose.

5 19. A method according to Claim 16 wherein said gel is polyacrylamide.

20. A method according to Claim 16 wherein said restricted DNA is stained with a DNA binding dye selected from the group consisting of ethidium bromide, propidium iodide, and fluorescent cyanine dyes.

10 21. A method according to Claim 16 wherein said at least one restriction enzyme is a 4 base cutter.

22. A method according to Claim 16 wherein said at least one restriction enzyme is a combination of at least one 4 base cutter and at least one 6 base cutter.

23. A method according to Claim 16 wherein said genomic DNA is from about 5 ug to about 30 ug.

15 24. A method according to Claim 16 wherein said resolving of restricted DNA at step (iii) results in the elimination of all DNA fragments less than 2 kb.

25. A method according to Claim 16 wherein said resolving of restricted DNA at step (iii) results in separation of DNA fragments over an distance of about 4 cm to about 12 cm of gel.

20 26. A method according to Claim 16 wherein said bacterial isolate is selected from the group consisting of *Escherichia*, *Salmonella*, *Shigella*, *Vibrio*, *Listeria*, *Staphylococcus*, *Streptococcus*, *Enterococci*, *Klebsiela*, *Nicericia* *Pseudomonas* and *Campylobacter*.

25 27. A method according to Claim 16 wherein said bacterial isolate is selected from the group consisting of *E. coli* 0157 and *Salmonella*.